

Final Notes from Meetings on
UPPER RESPIRATORY
DISEASE SYNDROME
in Desert Tortoises

October 12, 1989

U.S. Department of the Interior
Bureau of Land Management
1695 Spruce Street
Riverside, California 92507

and

October 13, 1989

Palace Station
Las Vegas, Nevada

Hosts: KRISTIN H. BERRY and SID SLONE
U.S. Bureau of Land Management

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IN DESERT TORTOISES

October 12, 1989: Bureau of Land Management, Riverside, CA

October 13, 1989: Palace Station, Las Vegas, NV

Agendas for the Two Meetings

Riverside. The meeting began about 8:30 a.m. with a greeting by Hugh Riecken, Assistant District Manager of the Bureau of Land Management's (BLM) California Desert District. The greeting was followed by introductions, with each person in the audience giving name, affiliation, and purpose for attending. A list of attendees is attached.

Dr. Kristin Berry reviewed the purposes of the meeting with the group and the group agreed on the agenda topics:

- (1) Review the existing data on the upper respiratory disease syndrome (URDS)
- (2) Review the existing data on health profiles of wild, healthy animals
- (3) Summarize what is known about the distribution and spread of URDS
- (4) Discuss and if possible reach a consensus for priorities for research, monitoring, publication, and disease prevention.
- (5) Focus on California for October 12 meeting:
 - a. determine degree of interest in participation from government agencies and private sector
 - b. determine potential availability of funds and budgets
- (6) Discuss special problems and topics
 - a. Future research on transmission **of** the disease, possibly at The Living Desert with experimental work under the supervision of Dr. Virginia Skinner
 - b. Salvaging genetic stock; American Honda project at the Desert Tortoise Natural Area
 - c. Desert Tortoise Natural Area
 - d. Quarantines
 - e. Proposal by off-road vehicle enthusiasts to feed and water tortoises in the Rand Mountain and Fremont Valley area

f. Prevention of disease

In addition, participants at the meeting will have opportunities to coordinate, get acquainted, combine efforts, and increase overall efficiency in dealing with URDS.

Las Vegas. The meeting began about 8:45 a.m. with a greeting by Ben Collins, District Manager of the Bureau of Land Management's Las Vegas District. The same procedures followed as described above, with introductions by participants. A list of attendees is attached.

Dr. Kristin Berry reviewed the purposes of the meeting with the group and the group agreed on the agenda topics:

- (1) Review the existing data on the upper respiratory disease syndrome (URDS)
- (2) Review the existing data on health profiles of wild, healthy animals
- (3) Summarize what is known about the distribution and spread of URDS
- (4) Summarize priorities for research, monitoring, publication, and disease prevention; review findings of Oct. 12 meeting
- (5) Focus on Nevada, Arizona, and Utah issues for the October 13 meeting:
 - a. determine degree of interest in participation from government agencies and private sector
 - b. determine potential availability of funds and budgets
 - c. discuss special problems and topics
 - quarantines
 - protecting genetic units, e.g., City Creek
 - prevention of spread of disease

In addition, participants at the meeting have opportunities to coordinate, get acquainted, combine efforts, and increase overall efficiency in dealing with URDS.

Presentation by Dr. Elliott Jacobson: Clinicopathologic Studies on an Upper Respiratory Disease of Desert Tortoises (Xerobates agassizi).

Authors: Dr. E. Jacobson and Dr. J. Gaskin

Dr. Jacobson and Dr. Gaskin are research and clinical veterinarians at the School of Veterinary Medicine, University of Florida, Gainesville. They specialize in research on reptiles and have additional specialties on viruses of captive reptiles (they have been very effective in solving some viral problems). They were awarded a

contract from the Bureau of Land Management (BLM) to start the process of identifying the pathogen(s) causing URDS. Funding for the project came from the California Department of Fish and Game and BLM.

Dr. Jacobson noted that URDS is widespread worldwide among several species of captive tortoises. It has been known for some time, especially in recently imported captives. The pathogenesis for URDS is yet undefined.

Relatively few diseases have been reported in free-ranging reptiles. Cutaneous fibropapillomas in sea turtles, which was first described over 50 years ago, is well established in green turtle populations in Florida and Hawaii. The cause is thought to be viral, but as of yet a virus has not been isolated. It is a very visible disease. URDS is also visible; with death the shell is also left behind.

In captive tortoises, the URDS can be chronic. Pet owners generally bring in the captive when the situation is severe (typical of pet owners in general). Dr. Jacobson has looked at many animals over the years. In captivity, tortoises can linger on for months, eating marginally and looking bad. Dr. Jacobson emphasized that tortoises can linger for long periods of time and can survive with tremendous organ damage for a long time before dying.

Dr. Jacobson has treated captive tortoises with URDS utilizing antibiotics and other therapeutic agents, but nothing has consistently worked. The disease is chronic and persistent, producing a slow death. None of the captives are getting natural foods. Why don't captive tortoises recover? Diet is possibly a factor. Diets in captivity are contrived, not based on good science. This is a topic we don't know much about. In the wild, the situation is somewhat different because of the lack of nutrients and water.

NOTE from Dr. Christopher Brand: The primary etiologic agent during the early or acute stage of disease may be absent and/or replaced by a secondary or opportunistic pathogen.

Internal problems are manifested in scutes, shell, and skin. With URDS, the tortoise may look depressed with eyelids closed and eyes depressed; nasal discharge, exudate, or bubbles, or a crust around the nares; lack of appetite; immune dysfunction; weight may be low with a slow wasting away. Sometimes the nares will appear dry, but when the tortoise is disturbed or picked up, the nose will bubble. By the time the bubbly nose is apparent, the tortoise is in the chronic stage of the syndrome. Comparisons of ill and healthy tortoises are in Table 1.

Subtle changes in behavior may occur before the runny nose appears.

URDS is known world-wide in tortoises. The disease is currently termed a syndrome, because we don't know if the same factors are operating in each case or the same pathogens are involved. URDS has been observed in leopard tortoises, Indian star tortoises, and radiated

Table 1. Comparison of signs of **URDS** in ill and healthy tortoises.

Ill	Healthy
nasal exudate, bubbles, <u>or</u> crusts of dried mud, exudate in nares: crust of dried mucous on forelegs; nares may be plugged	dry nose; no dried mucous in nares or on forelegs
sunken eyes	bright eyes
weight loss	normal weight
dull integument	shine to integument
can be lethargic, legs hanging loose	active; can pull tightly into shell

tortoises. The animal cannot be separated from its environment. Imported animals are stressed.

Tortoises are difficult to work with and to get out of the shell. Reptiles are generally not administered oral antibiotics since uptake of drugs from the gastrointestinal system cannot be predictably determined.

Antibiotics given by injection are painful and may put a tortoise off its feed. Once the tortoise goes off its feed, it gets into negative nitrogen balance and the immune function may be compromised. There are strong links between nutrition, nitrogen balance and immune functions. Identifying the pathogen(s) will be difficult.

We need to know more about the healthy, wild tortoise, e.g., normal anatomy, normal physiology, and normal histology. Such information is essential for interpreting disease processes.

Methodology

Dr. Jacobson then described the research undertaken since early May of this year. Large parts of the current research project were done by other people, a team effort. Twelve wild tortoises were used in the research: **8** clinically ill tortoises from the Desert Tortoise Natural Area (e.g., Kern Co., CA) and **4** presumably healthy tortoises from Ivanpah Valley (e.g., San Bernardino Co., CA). They weighed between 0.6 and 4.0 kg.

Dr. Jacobson discussed the importance of developing techniques to assess the health status **of** the tortoises. The tortoises were evaluated **using the** following tests:

- (1) Hematology: RBC, WBC, differential WBC, Hb, PCV, fibrinogen. These techniques are more laborious for reptiles than for mammals.
- (2) Serum biochemistry. **18** factors: glucose, **NA**, **K**, Cl, CO₂, BUN, uric acid, creatinine, Ca, P, ALP, SGOT, SGPT, total

- cholesterol, triglycerides, total bilirubin, albumins, globulins.
- (3) serum analysis of Vitamin A and E
 - (4) liver vitamin A and E
 - (5) heavy metals in the liver (selenium, copper, iron, lead, cadmium, and mercury)
 - (6) respiratory tract anatomy, histology, and ultrastructure
 - (7) pathology (work up on diseased animals)
 - (8) microbial isolation and serology
 - (9) liver lipid metabolism
 - (10) viral isolation, bacterial isolation, Chlamydia isolation
 - (11) lipids are an important issue. Lipidosis is seen in **some** turtles.

Dr. Jacobson discussed problems of quality control, not only for gathering the samples from the animal, but in storing the samples and then using standardized equipment and techniques for the analysis.

There were significant differences ($P < 0.05$) between healthy and ill tortoises for the following health parameters:

Table 2. A comparison of health parameters in ill and healthy tortoises.

Health parameters	Ill Tortoises	Well Tortoises
Hemoglobin (g/dl)	7.6 \pm 1 (anemic)	9.0 \pm 0.3
Na (mEq/l)	153 \pm 13	136 \pm 5
BUN (mg/dl) (seasonal changes)	100 \pm 70	6 \pm 1
Creatinine (mg/dl)	0.3 \pm 0.1	0.2 \pm 0
Phosphorus (mg/dl)	1.9 \pm 0.4	2.5 \pm 0.2
Total cholesterol (mg/dl)	305 \pm 170	83 \pm 20
BUN/creatinine (mg/dl)	313 \pm 174	31 \pm 7
SCOT (U/L) (good for evaluation of tissue dysfunction)	117 \pm 59	56 \pm 21

There were no significant differences in serum vitamin A and E. Vit A deficiency is known to cause health problems.

Heavy Metal Screens: Conducted at the University of Arizona, Tucson, by Dr. Carlos Reggiardo at the Veterinary Diagnostic Lab.

- Mercury levels were 10 fold higher in ill tortoises **than in** healthy. However, the mercury levels were still 10 fold below acute toxicity in mammals. Mercury levels may be affecting the immune system. The source of mercury is unknown and the physiological effect is unknown.
- Iron in ill tortoises was five times higher than in healthy tortoises. Iron levels may be a function of red cell break down. For ill tortoises, 1526 ± 679 ppm vs. 361 ± 64 ppm in healthy tortoises. Excessive amounts of iron have been seen histologically in livers of chronically debilitated reptiles, and may be a result of combined red-cell breakdown and/or inability to reutilize iron.
- Liver vitamin A, E (ug/ml), and selenium. **No** correlation between liver and serum levels. Vitamin A level for **ill** tortoises was 3 times that of healthy tortoises. Also, vitamin levels were 10-fold lower than for Arizona animals, but Dr. Reggiardo did not provide data on possible seasonal changes.

Anatomy and Morphology of the Upper Respiratory Tract

The methods for this portion of the evaluation included anaesthesia, euthanasia, decapitation (heads were sectioned at AFIP [Armed Forces disease lab in Washington, D.C.], where there is an excellent bone lab), and necropsy. Vascular castings will be made of the head. The following is a brief summary:

- (1) Tortoises have an unusual nasal cavity. In front of the eyes, there is a large cavity which may be important in thermoregulation (dissipating heat from brain). Most likely it **is** also important as a sensory structure in olfaction. Transverse sections show that the nasal cavity is continuous with the nasal passageways. The nasal passageway opens into choanae, roof of mouth.

The nasal cavity is large with dorsal nervous epithelium (probably chemoreception) and ventral mucous epithelium.

- (2) The trachea is bifurcated; lungs are in the darsal position.
- (3) The gastrointestinal tract and lungs adhere to one another.
- (4) Cephalic vasculature will be examined.
- (5) Gross anatomy of lymphoid structures were examined (acetic acid digestion of connective tissue illuminates lymphoid structures). GALT-gut associated lymphoid system. The cloaca has important lymphoid structures.
- (6) European studies indicated that the thymus was seasonally dynamic. On diseased animals, finding the thymus was

difficult; apparently some atrophy or regression. On well animals, thymus was easy to see. Thymic involution in reptiles most likely occurs during hibernation (immune system function drops).

- (7) Biopsies/gross anatomy of the dermal bone of the carapace. Can dermal bone be used to assess health?
- (8) pathologic investigation
- (9) The necropsies revealed relatively little coelomic lipid deposition. Sizable fat bodies were present subcutaneously in the axillary area. These fat bodies were smaller in ill than in healthy animals.

Manifestations of URDS in Upper Respiratory Tract

Slides of the head of ill tortoises. Total blockage of nasal cavity with exudate occurred in one ill tortoise. Thus, one might not see bubbling or exudate if blockage has occurred. The bubbling is like a chronic sinus infection; eventually the passageway and/or external nares may become blocked. With one exception, the disease was confined to the upper respiratory tract. In Dr. Jacobson's opinion, only in chronically or very ill animals will there be lung involvement. This may be more likely to occur in captive tortoises which may live longer with this disease.

Pathologic Studies

- One of the eight ill tortoises had a goiter. The thyroid was four times normal size and filled with colloid. Epithelium was hyperplastic.
- One of eight ill tortoises had nodules in lung; granulomas are common in captive tortoises in terminal stages of syndrome.
- At beginning stages, the disease is confined to the upper respiratory tract.
- There is a tremendous nasal cavity with differences in epithelium dorsally and ventrally. A myriad of glandular structures produce secretions onto the respiratory tract mucosa to keep it clean. Immunoglobulins may be produced locally in the upper respiratory tract.

Several layers of epithelium exist. The normal dorsal epithelium of the nasal cavity is ciliated with a basal layer. The normal ventral epithelium is mucosoid and ciliated with a basal layer. Healthy animals have sharp, clear borders in different types of epithelial cells.

- At higher magnification, diseased epithelium consists of only a basal layer; outer layers are absent because the whole

surface epithelium has been lost; ciliated layer is gone and mucous movement is affected; basal layer is inflamed. There is debris, inflammatory cells, and the epithelium is thickened. Tremendous inflammation and proliferation of basal epithelial cells occurs. Tissue is destroyed. Therefore disease becomes chronic.

- Heterophils are common in diseased basal layer early in the disease. Macrophages may be present. Electron microscopic studies are underway.
- The presence of eosinophilic and intra-cytoplasmic inclusions/bodies in epithelial cells were seen and may indicate viral presence. At AFIP, there will be some electron microscopic work undertaken to determine the nature of these structures.
- The effect is very diffuse, with disease throughout the upper respiratory tract. All ill tortoises had the same, well-defined lesions in the upper respiratory tract.
- It is difficult to believe that the tortoises could completely repair because inflammation and changes are diffuse. An array of bacterial organisms are growing in the nasal exudate and it is impossible to tell if any are involved.
- The acute phases of the disease were missed. The early part of the disease may occur in the first two weeks. We still don't know what has happened here.

Microbial Isolation and Serology

Chlamydia. Dr. Carlos Reggiardo at the University of Arizona, Tucson, has been isolating a Chlamydia-like organism from the nasal passageways of ill and healthy desert tortoises. He has isolated this microbe from California individuals, but further characterization is required. Dr. Reggiardo will collaborate with investigators at the National Animal Disease Laboratory at Ames, Iowa. Research veterinarians have not been able to isolate Chlamydia as of yet from samples sent by Dr. Jacobson. Dr. Jacobson has not seen Chlamydia in the tissues of ill tortoises.

Viruses. Viral isolation has been negative, but this is not surprising since the tortoises which have been examined have not been in the acute stage (first 1-2 weeks of insult) of the disease. A virus may have come in early and then disappeared, e.g., shipping fever in cattle. Also, examples of herpesvirus in green sea turtles. The presence of a virus has yet to be demonstrated.

Pasteurella testudinis. This organism has been isolated in both healthy and diseased tortoises. All eight ill tortoises and one of four healthy tortoise were positive for cultures. None of the Honda tortoises tested positive. We need better definition in tortoises.

Pasteurella is known to cause disease in other organisms, e.g., Pasteurella multocida causes snuffles in rabbits (see paper by Yue-Shoring Lu, S. P. Pakes, C. Stefanu. The contribution of Pasteurella in URDS is presently circumstantial. However, it may be involved and may take off when tortoises are stressed.

Summary

There is no doubt that this is a very significant and diffuse disease, resulting in death. It has the capability of wiping out an animal population. With the long period to reproductive maturity of 20 years and with breeding adults dying of disease, the situation is extremely serious. In addition to the disease outbreak at the Desert Tortoise Natural Area with possibly 10 to 60% of the population involved, die-offs are underway in Utah and northern Arizona. The oldest, longest living land vertebrate in North America is dying.

We know why the disease is difficult to treat now in captivity: because of the **loss** of normal surface epithelium in the upper respiratory tract and the severe inflammatory changes.

This is not a problem that just developed. A virulent pathogen could be involved with captive tortoises with introductions to wild populations. However, the resident tortoises had to be in a state of vulnerability, e.g., from altered ecology and nutrition, lack of rain. Nutrition is probably involved/linked. The tortoise may be an indicator species and more than likely is the tip of the iceberg with regard to animal populations throughout its range.

NOTE FROM DR. BRAND: While there is reason to believe that these tortoises are stressed from a variety of potential factors, and that stress often sets up stages for disease (e.g., shipping fever), this is not always the case, particularly with an introduced or exotic pathogen. Here, all that may be required is a "hot" pathogen and a susceptible host, although stress imposed on top of that could make the situation worse.

Future Research Needs

- (1) studies on transmission of the disease
- (2) normative data base--mineral/vitamin screens; nutritive evaluation; how plant communities have changed
- (3) broad microbial studies on ill tortoises
 - a. viral and bacterial cultures of upper respiratory tract; make another attempt at virus isolation
 - b. innuno-peroxidase staining, a powerful technique to demonstrate antigen/antibody presence, e.g., for Pasteurella testudinis and Mycoplasma
 - c. development of serologic test for exposure to P. testudinis and Mycoplasma; what is serology in the wild;

ELISA test for presence of circulating antibodies for P. testudinis and Mycoplasma

- (4) Pathologic studies
- (5) anatomical and functional studies of the immune system
- (6) measuring circulating corticosteroids; may give indication of health status
- (7) nutritional assessment of habitat; appropriate analysis of plants for fats, carbohydrates, proteins, fiber, and minerals
- (8) standardize techniques for gathering data on blood, nasal smears, etc.
- (9) climatology; changes in availability and distribution of precipitation

Questions from the audience (California and Nevada):

- **Is** it possible that there are pathogenic and nonpathogenic Pasteurella testudinis? **YES**. Biotyping?
- Does the disease recur? **YES**
- What is the efficacy of quarantine? Probably not effective, especially if environmental factors (stress through habitat degradation) are involved in the syndrome.
- What are the potential effects of air pollution? **E. J.** doesn't think it could be through water droplets. **URDS** is seen in gopher tortoises in Florida where air pollution is minimal.
- Dr. Stoecklin: could condition of animals be rated? **E.J.:** will try to develop system for rating. Lesions were all the same in the tortoises examined.
- What are costs to work up animals (Robert Smith)? **Is E.J.** interested in doing more? **E.J.:** takes 4 hours per tortoise; easier to do necropsy; hard to isolate all microbes; must budget time. **So** many people are involved and have contributed to the project. The contributions have allowed completion of many aspects, e.g., histology. But need to do more with electron microscopy (E.M.) It is difficult to do histology, E.M, and microbial isolation on the same animal because of limited amount of tissue. More detailed data are needed on additional tortoises with E.M. Need to do work on early (acute) stages of disease.

Resentation by Dr. Ken Nagy on Health Profiles of Well Tortoises

Dr. Nagy summarized some of his research on desert tortoises, starting with research on the physiology of tortoises at the Nevada Test Site between 1976 and 1977. Dr. Nagy's interests revolve around the fundamental question of how an animal survives in the desert. The Department of Energy has funded his research for the last 18 years. The following are key points:

- (1) Most tortoises were juveniles and Dr. Nagy was focusing on water balance. There is no such thing as typical--there is a lot of variation.
- (2) When tortoises emerge in spring, they are in a physiologically stressed state and moderately dehydrated. The bladder serves as an internal canteen. After hibernation the urine is concentrated: brown, viscous--a cesspool. The tortoise is osmotically stressed from salts.
- (3) Tortoises forage on annuals, which are high in protein, salts, and water, and low in energy. Water is stored in the bladder, along with salts. Tortoises only ate 4 to 6 species of annuals (highly digestible). They fill up on water, but are not getting enough energy.
- (4) Spring rainfall will be used to flush bladder. The bladder urine will be replaced with dilute fluid. Tortoises can drink up to 35% of their body weight.
- (5) Senescent vegetation is harder to handle, low in water, high in protein (but nitrogen content is dropping), high in fiber and salt.
- (6) With summer, tortoise estivates. As summer progresses, activity decreases, and urine is held in the bladder.
- (7) With summer rains, the tortoise will flush the bladder, returning osmotic concentrations to normal. The tortoise will be in positive energy balance and will use water to hydrate plants. The result will be a negative water balance, because the tortoise is foraging on dehydrated forage, thereby increasing salt load.
- (8) Summer annuals are eaten, with water content high.
- (9) With winter retreat, metabolic rate is low, and there is metabolism of stored fat.

CONCLUSION: Tortoises abandon maintenance of homeostasis on minute to minute/day to day basis but maintain it on annual basis. Use of summer rainfall is crucial to the tortoise.

Health Profiles for Wild Tortoises - a normative database for California

Dr. Nagy and students Charles Peterson and Brian Henen **are** under contract to the Bureau of Land Management (BLM) to collect data on blood parameters, nasal mucous, fecal parasites, and shell scrapings from 60 wild tortoises at four times during 1989. No bone biopsies have been taken. The tortoises all have radiotransmitters and are on three study plots, with 20 (10 adult males, 10 adult females) at each of three sites: the Desert Tortoise Natural Area interior, Ivnpah Valley, and Goffs. The BLM added to existing graduate research projects undertaken by Charles Peterson and Brian Henen--a piggyback arrangement. Dr. Nagy will be collaborating with Dr. Walter Rosskopf for interpretation **of** the findings. Dr. Nagy and students have undertaken this special effort without salary. **If** funds are available, the project will be continued in 1990.

Ongoing Studies - graduate students

- (1) Chuck Peterson: comparative energetics and water balance in western and eastern Mojave populations of the desert tortoise
- (2) Brian Henen -fat and reproduction in southeastern Mojave Desert tortoises.

Dr. Nagy **is** interested in conducting field nutritional studies of nitrogen digestion, assimilation. Also wants to determine chemical composition **of** plants tortoises do and don't eat. Some of material will be applicable to research on translocation of tortoises in the future: do they eat enough?

Proposed Studies - team of experts at UCLA, including Dr. Fred Turner (advisory capacity), Hal Avery (amino acid nutrition), Dr. Mark Wilson (wants to come and work on stress hormones), student Paul Stone (wants to come), Dr. R. Tracy (visiting scientists, working on digestion).

A wide range **of** physiological and ecophysiological and endocrinological studies are anticipated.

Question: What is the role **of** hydrocarbon or other pollutants on energetics and water balance? NOT **KNOWN**.

Question: What impact is there of changes in species composition and is there any relationship between ill and healthy populations in plant species utilization? NOT KNOWN.

Presentation by Dr. Cecil Schwalbe on Physiological Characterization of Health in Desert Tortoises - A Normative Data Base

Several government agencies in Arizona and Utah have combined efforts and funds to develop a normative database on healthy and diseased wild tortoises. Tortoises from different habitats are examined physiologically by Dr. Jim Jarchow and Dr. **E.** Jacobson using nasal swabs, blood samples and bone biopsies (technique from Dr. Tom Wronski/E. Jacobson). X-rays are also taken for assessment **of** shell thickness. The expectation **is** to characterize status of bone, e.g.,

changes or growth as a mechanism for assessing environmental impacts.

Seventeen tortoises have been fitted with radiotransmitters at the Beaver Dam Slope in Arizona and the City Creek site in Utah (a thriving population with about 400 tortoises/mi²). The two sites allow a comparison between grazed and ungrazed habitats.

This work, Dr. Nagy's efforts in California, and Dr. Jacobson's concerns all point toward the requirement for standard protocols.

Question: Are any data available on population estimates? Yes. Study sites are BLM plots and were previously censused using 60-day surveys.

Question: Any indication of URDS in Arizona? The topic will be addressed later.

Distribution and Spread of URDS in the U.S.

California. In California the URDS is present at several sites (Figure 1) in the western Mojave Desert.

Desert Tortoise Natural Area and Nearby Areas. In 1988, Charles Peterson reported signs in 25% of his research animals at the Desert Tortoise Natural Area, and about the same time Dr. Craig Knowles, a BLM contractor, reported observations on the Desert Tortoise Natural Area interior study plot, where 168 tortoises were registered. Dr. Knowles reported that about 8% of the tortoises he observed in the latter part of the survey had signs.

In spring of 1989, Dr. Craig Knowles et al. of FaunaWest conducted a survey in the Desert Tortoise Natural Area, Rand Mountains, and Fremont Valley on a BLM contract. Four hundred sixty-eight individuals were sampled and 43% showed signs of URDS. The actual frequency of URDS may be higher, because signs are not always obvious. Dr. Knowles reported that his team increased in proficiency in detecting signs as the field season progressed. The frequency of signs in adult tortoises was higher (46%) than in juveniles (26%). Dr. Knowles also resurveyed the Desert Tortoise Natural Area interior study plot and found that 20% of the 168 tortoises observed alive in 1988 were dead. Most of the dead animals were large adults. This estimate is likely to be low because the survey was cursory and tortoises can die underground too. NOTE: Berry says the figure for dead animals is now higher. Ordinarily, about 1-2% of adults die annually. About 52% of live tortoises (N = 31) had signs of URDS, a substantial increase from the previous spring.

In spring of 1989, the BLM also conducted a survey of the 3-mi² interpretive center plot at the Desert Tortoise Natural Area. Of the 216 tortoises in the sample, about 9% showed signs.

Honda Properties. Dr. Mike Weinstein and Peter Woodman reported 1 to 2 freshly dead tortoises out of about 80 to 100 live tortoises at the American Honda property (adjacent to the northwest corner of the Desert

Tortoise Natural Area). Four sick tortoises also have been found.

Edwards Air Force Base. Tim Shields (pers. comm.) reported that two out of four tortoises observed in an area of about two square kilometers showed signs of URDS.

Peter Woodman provided data from several western Mojave sites. He, Steve Boland, Gilbert Goodlett and Jeff Kaufmann have conducted calibration transects on three BLM permanent study plots (Fremont Peak, Stoddard Valley, Lucerne Valley) as well as walking transects elsewhere (Lenwood, and Ft. Irwin). The following are data from Woodman:

Stoddard Valley study plot. The BLM's Stoddard Valley plot was surveyed by a BLM contractor (Dr. Craig Knowles) in spring of 1987. Dr. Knowles, in recalling his field observations during that year, believes that he saw at least one tortoise, a large male, with signs of URDS. in summer and fall of 1989, Woodman reported seeing 10 live tortoises during 7 to 8 days of surveys. Eight of the 10 live tortoises showed signs of URDS. He also observed six recently dead tortoises (four of which had been shot).

Lucerne Valley study plot. Woodman reported seeing eight live tortoises during four days of surveys in August, September, October, and November of 1989. Of the eight, three showed signs of URDS. He also saw four recently dead tortoises (1 immature, 3 adults). The BLM last conducted surveys of this plot in 1986 and have plans to re-survey the site in spring of 1990.

Fremont Peak study plot. This plot was surveyed by Dr. Knowles in spring of 1989. Dr. Knowles reported finding two out of 29 tortoises with dirt caked in the nostrils. During summer and fall of 1989, Peter Woodman observed three live tortoises, none of which showed symptoms of URDS. He found two carcasses (both adults, one marked) of tortoises which appear to have died since spring.

Ft. Irwin. During 1988-89, Peter Woodman conducted strip-transects at Ft. Irwin National Training Center and to the south. During July and August of 1989, he observed 8 live tortoises, none of which showed signs of URDS. He also saw two to three recently dead tortoises, crushed by tanks.

Lenwood. A site about 7 miles south of Barstow with country club and 9-hole golf course. During surveys conducted on July 5 and October 1-3 of 1989, Woodman saw a total of 13 live tortoises, of which 2 showed signs of URDS. The two ill animals were found in October, within 100 yards of each other. Woodman also saw one recently dead tortoise. Woodman stated that this area still had good densities of tortoises.

In the western Mojave Desert of California, tortoises with signs of URDS have been found on both sides of 1-15, Hwy 58, Hwy 395. The disease appears widespread, with only the area north of Barstow apparently free of symptomatic tortoises.

Fenner - Chemehuevi population. Two tortoises with possible signs of URDS were observed in the Fenner-Chemehuevi habitat September 11-16, 1989 by government employees surveying the California loop of the SCORE Parker 400 off-road vehicle race (Mike Henderson, Jennifer King, Bill Grossi, Jerry Page, and Jeff Krausmann). A report by M. Henderson and J. King describes a large male (265 mm carapace length) walking on desert pavement and wheezing. The report also contained notes on a 201 mm subadult/adult which had one nostril plugged. Reference: Henderson, M. and J. King. 1989. Survey of the Parker 400 race course within categorized desert tortoise habitat in California. September 11-16, 1989. Bureau of Land Management, Havasu Resource Area, Yuma District, Arizona.

Chuckwalla. No tortoises with signs of URDS have been observed in the Chuckwalla habitat, although the Chuckwalla Bench has experienced a major dieoff since 1982 from a second but unidentified disease.

Question: **Why** have no sick tortoises been reported from other areas?
More intensive surveys have been conducted in the western Mojave; more surveys have been conducted for private entities and government agencies. Additional surveys should be undertaken in other parts of the State.

Nevada. Brad Hardenbrook, Nevada Dept. of Wildlife, reported that several tortoises with possible signs of URDS have been reported from sites to the west and north of Las Vegas: (1) at Summerlin, one tortoise collected in September of 1989 was diagnosed with URDS; (2) the Kerr-McGee industrial park (Apex; about 30 live tortoises and 47 carcasses were found on 280 acres); (3) proposed Bonneville Pacific Cogeneration Site (at least two and possibly three tortoises showed signs of URDS in summer of 1989); (4) two tortoises out of 50 or 60 at Mormon Mesa showed signs in spring of 1989 (Figure 2). Phil Medica reported observing one tortoise with a runny nose on the Nevada Test Site in 1987.

Question: What happened to sick tortoises? One was seized by the Nevada Department of Wildlife. The remainder are still in the wild.

Question: How much effort has been put into searching for tortoises with disease? Not much. Previous surveyors may not have known what to look for.

Question: Were 47 carcasses or 47 shells found? Most remains were shells.

Arizona. Cecil Schwalbe provided a map (Figure 3) of five sites where tortoises have been diagnosed with URDS from 1987 through 1989. Ten of 59 tortoises inspected in 1989 on the Beaver Dam Slope in Arizona presented signs of URDS. In 1987, three of 49 tortoises on the Littlefield Plot portion of the Beaver Dam Slope (Arizona) presented signs of URDS. (These figures do not include tortoises on the Utah

portion of the Beaver Dam Slope).

In the Sonoran Desert, tortoises with signs were found at four sites: Avra Valley (N = 3 of 13, 1987), Galiuro Mountains (N = 1 of 30, 1989), Santa Catalina Mountains (N = 4 of 14, 1988-89), and in Saguaro National Monument East (N = 4 of 28, 1989). Of 144 tortoises in the combined sample for Arizona, 22 had signs of URDS (1 died). Overall, 15% of tortoises showed signs of disease in areas where disease occurred. Several sites had tortoises with no signs, but those sites were not named or included in the computations. Additional sites may be surveyed in 1990.

~~Utah~~. Both John Payne and Tim Duck gave reports during the two-day meeting. Tortoises on the Beaver Dam Slope were first studied in the 1930's by Woodbury and Hardy. In 1977 Eric Coombs reported tortoises with URDS. The Utah Division of Wildlife Resources had a captive-release program for several years and at least two sick captives were released. Densities on the study plots have decreased markedly over the last several years. In 1986, 8 of 20 tortoises on the BLM's Beaver Dam Slope plot showed signs. In 1988, the Division of Wildlife Resources had radiotransmitters on 21 tortoises. By 1989, 12 (or 7?) of these were dead. One was sick September 7 and died by September 12. Three of five tortoises found south of the Beaver Dam Slope plot (when?) were also reported to have signs.

According to Tim Duck, no tortoises with signs have been reported from the City Creek study plot, a site with a high density of tortoises. However, some tortoises (two of 30) with URDS have been found near the plot. Todd Esque found one possibly symptomatic tortoise here.

Question: Are there any differences in occurrence of disease in or out of the exclosure? No observed difference.

Question: Is there a habitat difference between City Creek and the rest of the Beaver Dam Slope? Yes. City Creek has not been grazed in 10 to 20 years and has less impacts. Soils are different. City Creek is in sandstone, whereas Beaver Dam Slope is in creosote-Joshua tree woodland with caliche washes.

General Question: Is removal of diseased animals worthwhile and beneficial as a preventive measure? One Answer: Not practical; may not reduce incidence of URDS, may artificially reduce reproductive potential of population.

Dr. Jacobson not comfortable with this answer. Sick tortoises bother him: their reproductive potential *is* often low. Also, potential to infect many tortoises, e.g., female prostitute with AIDS in the Phillipines. CONCLUSION: WE WILL NEED TO REVISIT THE ISSUE OF REMOVING SICK TORTOISES.

Institutions and Individuals Interested in Participating in

Future Research on URDS- Discussion on October 12

Dr. Kurt Snipes and Dr. Tim Carpenter, University of California at Davis. See attached letter. They are interested in use of simulation models to predict impacts.

Fish and Wildlife Service. Some participants expressed a desire to have the Service fund an effort to standardized field forms and methods of observations for recording symptoms of **URDS**.

Col. George Lewis. The military and Defense Department are interested in **URDS** at the highest levels of the pentagon and can provide extensive laboratory support for appropriate **URDS** investigations. The U. S. Army Medical Research Institute of Infection Diseases (USAMRIID), Ft. Detrick is interested in collaborative research with interested parties. No cash will be involved, just staff, infectious disease expertise, research, and lab assistance.

Mary Lamb. The U.S. Air Force has no money, but installation commanders are instructed to consider threatened and endangered species conservation and to budget accordingly. There is a Defense National Resources Council; Christina Ramsey chairs the Council, which had a \$400,000 budget in FY90.

Roger Twitchell. The U.S. Marine Corps is interested in providing support for research rather than consultations with the U.S. Fish and Wildlife Service. A discussion ensued on how the two topics were not interchangeable.

Dr. Virginia Skinner, veterinarian at The Living Desert, described the zoo and botanic garden, which has exhibits of North America and the world deserts. Veterinary facilities include a clinic, holding facilities, and five keepers.

Dr. Skinner offered the facilities of The Living Desert for research on transmission of **URDS**, and talked about working with Dr. Jacobson. Koch's hypothesis should be satisfied with the experiments. For example, an infected tortoise could be introduced to a group of six healthy tortoises, with controls. Healthy tortoises could be inoculated with filtered and unfiltered (millepore filter) exudate. Experimental animals and controls should be maintained on a natural diet of vegetation to eliminate stress.

Issues include sizes of pens. How has American Honda solved the pen problem?

People Interested in Research on Disease Transmission:

Dr. Dave Jessup, California Dept. of Fish and Game
Dr. Keith Dupre, University of Nevada at Las Vegas
Dr. Virginia Skinner, The Living Desert

Dr. Elliott Jacobson, University of Florida
Betty Burge, Las Vegas
Dr. Tim Carpenter, University of California at Davis
Dr. Kurt Snipes, University of California at Davis
Dr. Christopher Brand, Fish and Wildlife Service, NWHRC

Institutions and Individuals Interested in Participating in

Future Research on URDS- Discussion on October 13

Dr. Keith Dupre. Can contribute in general area of respiratory physiology, as well as ecology. Dr. Dupre is interested in how the disease affects health of tortoises, e.g., blood gases. Dr. Joe McCarlluf is working in the field of reproductive physiology and ecology radio-isotope dating of mortalities (very expensive). He has worked with other species of tortoises.

General Comment: Phil Medica said that mortality in dry years or poor forage years can be high without the presence of disease in tortoises. He referenced a BLM research project (published in Copeia) in Ivanpah Valley where losses of adult tortoises were high in both 1980 (4.4%) and 1981 (18.4%). The 1980-81 losses appeared to be correlated with weather. Fieldworkers should be cautious about associating high mortality rates only with URDS. Medica predicts a high mortality rate at Ivanpah in 1990, if dry weather continues.

Col. George Lewis. The Defense Department has 9 laboratories in the USAMRIID at Ft. Detrick, MD, which has a staff of approximately 500. The USAMRIID has biological containment facilities and conducts research on highly infectious agents. The laboratory has a concentration/collection of virologists, microbiologists, pathologists, etc.

Robert Smith, USFWS. \$125,000 of Service monies were transferred to University of Arizona Coop unit. Additional staff may be hired.

Brad Hardenbrook, Nevada Department of Wildlife. Some limited disease surveys will take place in 1990, but it is too early in the budget process to predict.

Les Monroe, Department of Energy, Nevada Test Site. Long-term monitoring and funding are uncertain.

Kent Turner, National Park Service, Lake Mead. Surveys anticipated from Davis Dam to Eldorado and River Mountains, but funding is uncertain.

Paul Selzer, Clark County Habitat Conservation Plan. Urban areas in deserts can be sources of funds for research and land acquisitions through mitigation fees. This will involved permitted incidental take in Las Vegas subunit.

Gene Dahlem. BLM, Arizona. Funding unclear for inventory and monitoring in four districts.

Dr. Cecil Schwalbe, Arizona Game and Fish Department. Anticipate four 60-day surveys of study plots; also sampling for health profiles with assistance of Veterinary Diagnostic Lab at University of Arizona.

John Payne, BLM, Utah. Reproductive study funded by Utah Division of Wildlife Resources will continue. Some monitoring.

NOTE FROM: **Dr. Christopher Brand**, U.S. Fish and Wildlife Service, National Wildlife Health Research Center. The Center has P-3 diagnostic and research laboratories and animal isolation facilities. Staff includes pathologists, microbiologists, virologists, and epidemiologists.

Special Subjects for Arizona, Nevada, and Utah

- Issues:**
- (1) Quarantine
 - (2) Short-term actions to preclude extinction
 - (3) release of captives and development at City Creek
 - (4) Protocols for wild, symptomatic tortoises
 - (5) public education/public relations - release of captives

Quarantine. Tom Ballow, Nevada Department of Agriculture. Who has quarantine authority? The Nevada State Quarantine Officer has no control unless the animal is under the control of man (a captive/domestic). The following were several key points:

- Evidence of transmission of **URDS** from captives to wild populations is circumstantial.
- Release of captives to the wild is a violation of Section 9 of the Endangered Species Act; should the Quarantine Officer bring sanctions against captive releases also?
- A quarantine will be challenged
- Committee to deal with public education: no support from audience
- TV and radio may be most effective media for public education

List of short-term actions. A list of tangible products resulting from this meeting:

- Fencing
- Protocols for visual assessment of health (USFWS)
- Protocols for handling tortoises, sampling blood and other tissues, and preserving and analyzing specimens (USFWS)

Management of City Creek.

- Fence it all or fence approaches/access
- Target a tortoise drop-off site.

Captive Problems in General. Significant problems include: the lack of resolution of the status of captives, escaped captives, off-spring of captives, and free-ranging tortoises on private land.

Long Term Actions (Research) vs. VS. Immediate Actions**Discussions from California Meeting**

Immediate Needs. Budgets must be identified before short and long-term actions and research can be prioritized.

- (1) Etiology and descriptive epidemiology must be understood.
- (2) Standardized protocols should be developed to educate people about their sick pets and to inform them of responsible options (turn in tortoises to Wildlife Adoption Programs, Vets, etc.)
- (3) Funds must be raised to support education program.
- (4) Use Research Packet developed by the four states (Rangewide Funding for Proposed Research, July 1989) as a starting point
- (5) Needs for meetings - options
 - small sub-Committee meetings can handle decisions prior to next large forum on disease
 - Use 1990 Desert Tortoise Council meeting to discuss disease issues

Long-term Considerations:

- (1) Habitat protection
- (2) Propagation of genetic stocks
- (3) Determine link of nutrition with disease
- (4) transmission studies
- (5) prospective epidemiology studies (separate from descriptive epidemiology)

General Discussion on Above Points.

- Captive breeding may be a solution for restocking populations. Some females with **URDS** are able to produce viable eggs.

- Zoos or The Living Desert may be suitable sites for captive breeding. In Las Vegas Valley, TORT Group wants the Habitat Conservation Plan to include a holding/breeding facility.
- Fresno Zoo is establishing an exhibit with long-term captive family.
- Captive breeding raises the research issue of relocation and its feasibility.
- It may be useful to have US Fish and Wildlife Service draft captive breeding protocols.
- Central repository for data
- Laboratories for processing blood and other tissue specimens should be identified.
- Quality Control. Efforts and methods should be coordinated and standardized as much as possible.
- Should field workers have standardized training? (sampling, describing clinical signs, recording observations, etc.) Perhaps a protocol or short course.
- BLM policy in terms of habitat and population preservation may be stronger than the US Fish and Wildlife Service and Endangered Species Act.
- Ravens should be considered for removal from the Migratory Bird Treaty Act

SUMMARY DISCUSSION

Short-term Goals:

Establish protocols for:

- recording field observations of healthy and sick tortoises
- forms for recording data
- Protocols for sampling and analyzing blood, nasal swabs, etc. Dr. Jacobson noted that there **is** no quality control for animal samples, such as is available for human tissues (American Pathologists have a quality control **system**) . Machines can differ in results. Results of data should be compared from one lab to another.

Standards for blood such include (1) how and where to take blood from a tortoise, and (2) serum should be drawn off and frozen in 45 minutes.

Standards for nasal swabs should consider (1) trauma to mucosa (consider small catheter with syringe and carry sterilized packs); (2) isolates may be different if collected from choanae **vs.** nares

- draft captive breeding protocol
- prepare protocol for training field biologists
- review protocols annually for revisions
- consider centralizing data base

Long-term Goals.

- (1) identify pathogen(s) involved in causing URDS
- (2) determine how URDS is transmitted
- (3) determine epidemiology
- (4) determine health profiles of well, wild tortoises
- (5) determine the role of general health and nutrition in URDS

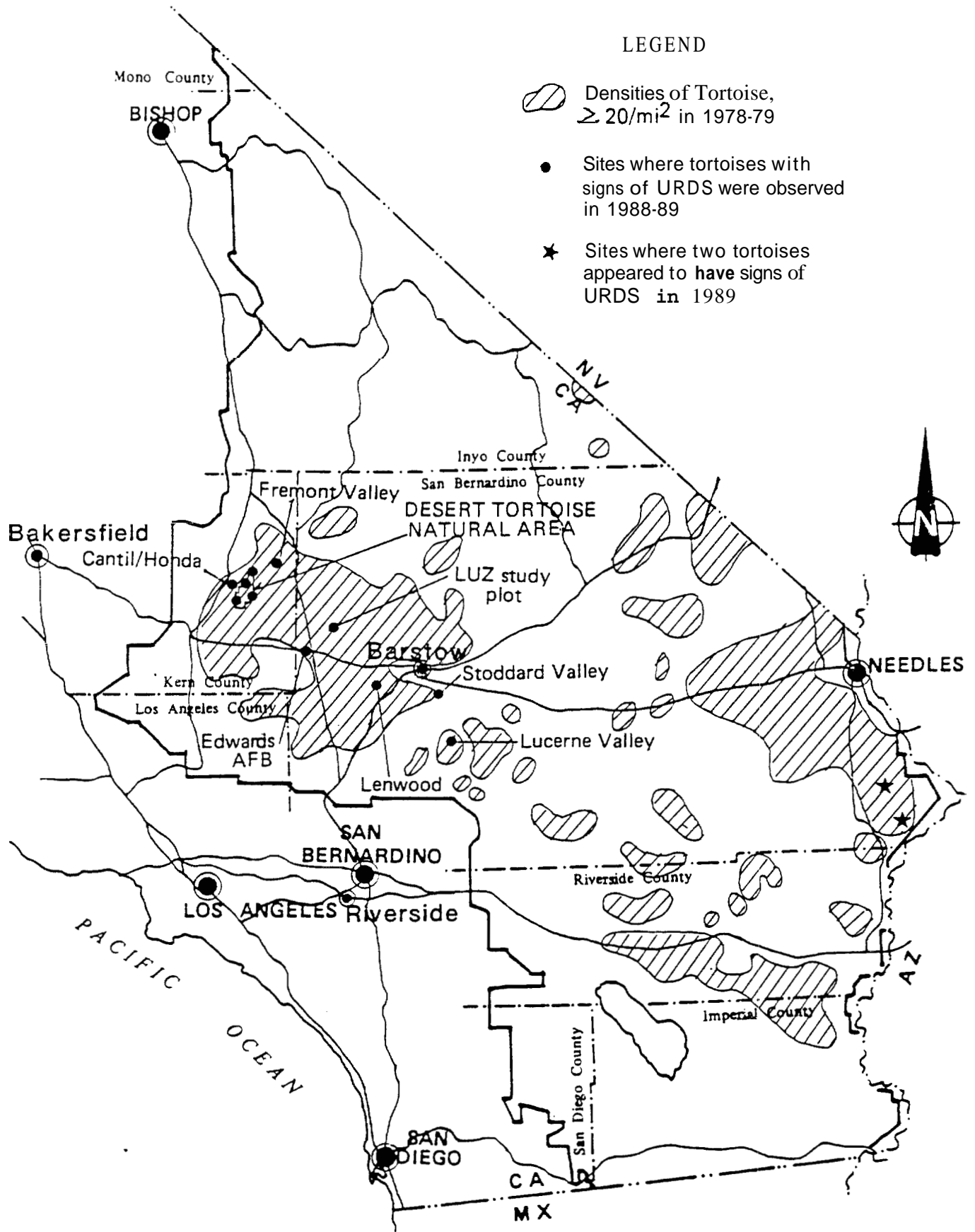


Figure 1. Locations of desert tortoises with signs of upper respiratory disease syndrome in the California Desert Conservation Area.

Phil Medica observed
one tortoise with a
runny nose - 1987

ROCK VALLEY



MORMAN MESA

Study Plot



Two tortoises in Spring
1989 had signs of URDS.
One had a runny nose;
the second had a wet
face and unclear eyes

Proposed Bonneville
PACIFIC
Cogeneration
Site



At least two, and possibly
three tortoises showed
signs of URDS in summer
1989
(Gilbert Goodlett)

SUMMERLIN



One tortoise removed
Sep. 1989, was
diagnosed with URDS

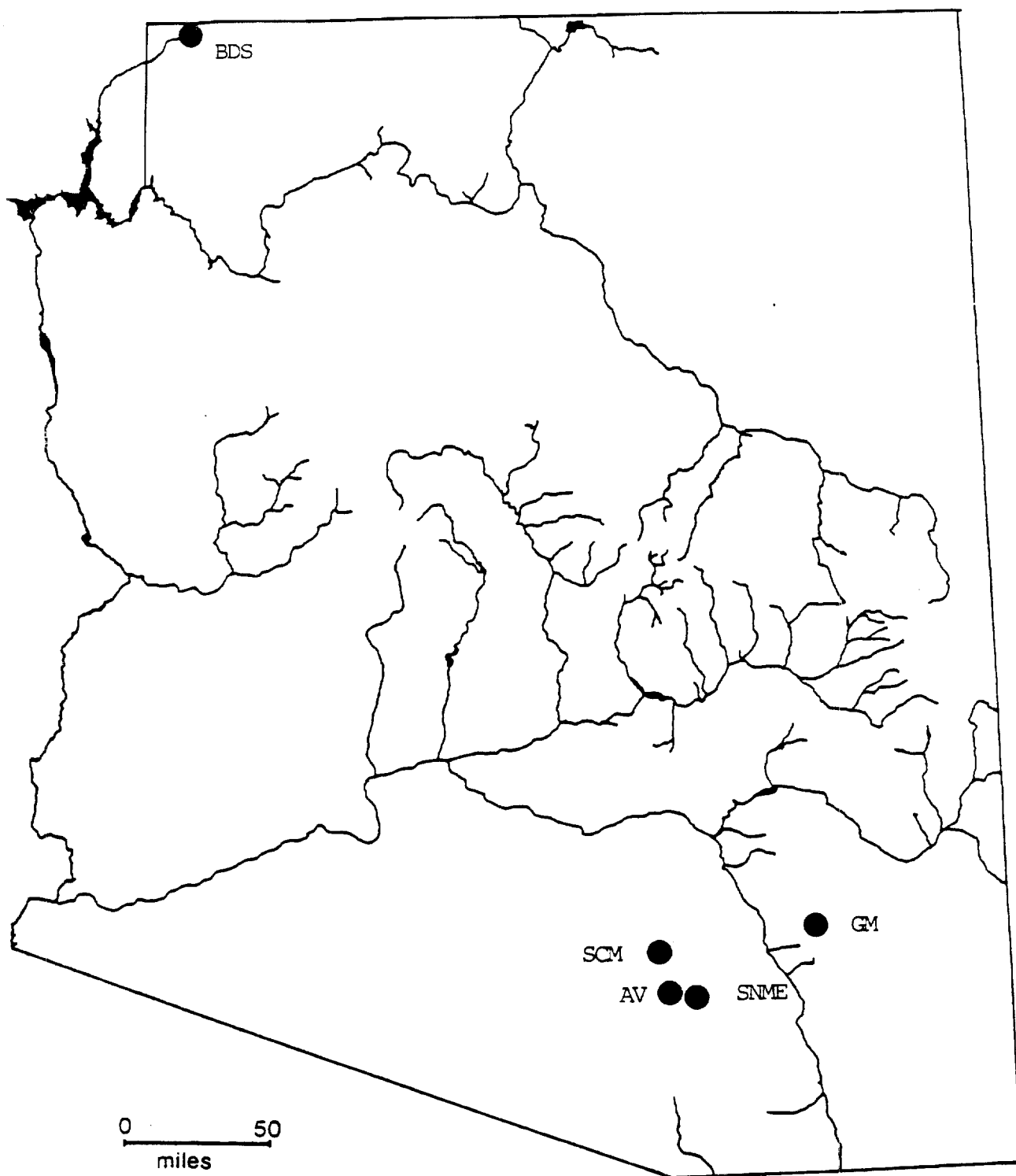


NV

Figure 2. Locations of desert tortoises with signs of upper respiratory disease syndrome in Nevada.

Figure 3. Locations of wild desert tortoises with signs of upper respiratory disease syndrome in Arizona.

URDS* IN ARIZONA DESERT TORTOISE POPULATIONS
1987-1989



*Upper Respiratory Disease Syndrome

AV	= Avra Valley	3/13	1987
BDS	= Beaver Dam Slope	10/59	1989**
GM	= Galiuro Mountains	1/30	1989
SCM	= Santa Catalina Mountains	4/14	1988-89
SNME	= Saguaro National Monument East	4/28	1988-89

Numbers indicate the number of tortoises **with** respiratory disease signs over total tortoises observed, followed by year(s) in which observations made. **Does not include three of 49 tortoises found with URDS on the Littlefield Plot in 1987.

LIST OF ATTENDEES AT THE MEETING ON THE
UPPER RESPIRATORY DISEASE SYNDROME IN DESERT TORTOISES

October 12, 1989

Bureau of Land Management, 1695 Spruce Street, Riverside, CA

Veterinarians/Contractors

Dr. Elliott Jacobson, Health Sciences Center, Box J-6, College of
Veterinary Medicine, University of Florida, Gainesville, Florida
32610

Dr. Virginia Skinner, The Living Desert, 47900 Portola Ave., Palm
Desert, CA 92260

Dr. Kurt Snipes, School of Veterinary Medicine, Dept. of Epidemiology
and Preventive Medicine, University of California, Davis, CA 95616

Dr. Tim Carpenter, same as for Dr. Snipes

*Dr. H. J. Holshuh II, Laboratory and Disease Investigation, Comparative
Medical and Veterinary Services, Dept. of Health Services, County
of Los Angeles, 12824 Erickson Ave., Downey, CA 90242
(213)-940-8801.

University of California, Los Angeles and other Universities

Dr. Ken Nagy: Laboratory of Biomedical and Environmental Sciences, 900
Veteran Ave., Los Angeles, CA 90024-1786

Chuck Peterson: same as above

Brian Henen: same as above

Dr. Bill Shaw, School of Renewable Natural Resources, University of
Arizona, Tucson 85721 (602-621-7265)

Dr. Keith Dupre, Department of Biological Sciences, Univ. of Nevada, Las
Vegas 89154

Bureau of Land Management

Dr. Kristin Berry, BLM, 1695 Spruce Street, Riverside, CA 92507

Curtis Warrick, BLM, Arizona Strip District, 390 North, 3050 East, St.
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Tim Duck: BLM, Shivwits Resource Area, 225 N. Bluff, St. George, Utah
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Gary Ryan, BLM, same as for Slone

John Payne, BLM, Dixie Resource Area, 225 N. Bluff, St. George, Utah
84770

U.S. Fish and Wildlife Service

Robert Smith, USFWS, 1002 NE Holladay Street, Portland, Oregon
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Mary Jo Elpers, USFWS, 4600 Kietzke Lane, Bldg. C, Reno, NV 89502

Judy Hohman, USFWS, Ventura Field Office, 2140 Eastman Ave., Suite 100,
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Dr. John Oldemeyer, USFWS, Ft. Collins, CO (Same as for Schamberger)

National Park Service

Robert Moon, Joshua Tree National Monument, 74485 National Monument
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Ken Turner, Lake Mead National Recreation Area, 601 Nevada Hwy, Boulder
City, NV 89005

Department of Defense

Roger Twitchell, Installations Division, Natural Resources/Environmental
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Lt. Col. Martin Crumrine, Bacteriology Division, USAMRIID, Ft. Detrick,
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ATTN: AFZJ-EHE-SP, National Training Center, Ft. Irwin, CA
92310-500
Lt. Col. John Wright, Director of Engineering and Housing, same as for
Lt. Buckrucker
Mary Lamb, Air Force, AFRCE-WR/ROV, 630 Sanson Street, San Francisco, CA
94111
*Doug Pomeroy, Dept. of Navy, Western Division, Naval Facilities
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Christine Bates, Chocolate Mountains, Marine Corps Air Station, Public
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"Dr. Jerry Boggs, Naval Weapons Center, Environmental Branch, Code 2662,
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California Department of Fish and Game

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John Brode, California Dept. of Fish and Game, Inland Fisheries
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Brad Hardenbrook, Nevada Dept. of Wildlife, State Mailroom Complex, Las
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Betty Burge, TORT Group, 5157 Poncho Circle, Las Vegas, **EN** 89119

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Dr. Ver Steed, Upjohn Company, **Route** 4, Box 205B, Porterville, CA 93257
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Paul Fromer, RECON, 1276 Morena Blvd., San Diego, CA 92110
(619-275-3732)

*Not present, but will send notes

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October 13, 1989
Palace Station, Las Vegas, Nevada

Veterinarians/Contractors

Dr. Elliott Jacobson, Health Sciences Center, Box J-6, College of
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Dr. Gary Weddle, Black Mountain Animal Hospital, 1000 South Boulder
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Dr. George Stoecklin, 2437 East Cheyenne, North Las Vegas, NV 89030
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University of Nevada and other Universities

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Tim Duck: BLM, Shivwits Resource Area, 225 N. Bluff, St. George, Utah
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Mark Maley, same as for Slone (702-646-8899)
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Judy Hohman, **USFWS**, same as for Peter Stine.
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Bob Tronstad, DVM, Domestic Animal Diseases (702-789-0185); same as for
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Nancy Nicolai, RECON (619-275-3732), same as for Paul Fromer

Caryn Shetterly, Las Vegas Review Journal (702-383-0308)

Philip A. Medica, Reynolds Electric and Engineering Co., P.O. Box 495, Mercury, NV 89023 (702-295-7114)

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G. Scott Mills, SWCA, Inc., 1602 E. Fort Lowell, Tucson, AZ 85719 (602-325-9194)

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*Did not attend, but sent notes